SPONTANEOUS AMEBIC DYSENTERY IN MONKEYS*

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This article records a spontaneous outbreak of a disease in monkeys in which the lesions corresponded closely to those found in amebic or tropical dysentery in man, and in which protozoal organisms occurred that had the structure and characteristics of those amebas generally considered the causative agents in human tropical dysentery.

This spontaneous outbreak of amebic dysentery is of special interest since there appears to be no record of a similar case, and because our knowledge of this disease in animals is very meager. It is probable that the affection was introduced with one or several of the imported monkeys, and conveyed to the healthy individuals kept in the same cage. The possibility of transmission of the ameba to human beings through such sources must be given cognizance, and, furthermore, our findings suggest that at times imported animals might be carriers of the parasites without disclosing any clinical evidence. This phase of the manner in which disease may be spread is now continuously gaining in importance.

Since the first ameboid protozoan was discovered by Roesel[1] (1755) numerous investigators have isolated this type of organism from various sources, differentiated the genus into a number of species, both parasitic and nonparasitic, and have attempted to classify the former as pathogenic and nonpathogenic. Lambl[2] (1859), Loesch[3] (1875), and Kartulis[4] (1885) observed amebas in fecal matter from dysentery patients, but Koch[5] (1887) demonstrated amebas in tissues undergoing ulceration, and was the first to establish a connection between amebas and the lesions of the disease known as tropical dysentery. Later investigators have confirmed the association of the parasites with dysentery, and have considered them the causative agent in this disease. Others have disputed the pathogenic rôle of amebas, asserting that the organisms can be demonstrated in the intestinal contents of a large percentage of men and animals in normal condition.

Tho the question of pathogenicity, and of permanent or facultative pathogenicity of certain species is undetermined, it is apparent from

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[1] Insecten-Belustigung, 1755, 3, p. 101.

[2] Beobachtungen und Studien aus dem Gebiete der pathologischen Anatomie und Histologie, Teil 1, 1860.

[3] Virchow's Arch. f. path. Anat., 1875, 65, p. 196.

[4] Ibid., 1885, 99, p. 145.

[5] Arb. a. d. k. Gsndhtsamte, 1887, 3, p. 13.

the specific character of the lesions in which these organisms are found that they are an important, if not the only, etiologic factor in the pathologic processes.

There are few data available on the spontaneous infection of animals. Artificial infections have been successful with various species of animals, especially with cats. However, in order to obtain such results material containing the spores or the encapsulated forms of amebas had to be fed, since the vegetative forms were invariably destroyed in the animals' stomachs. Spontaneous amebic dysentery complicated with liver abscess has been observed in a badger (Meles taxus) in the zoological garden at Cairo, and according to Strong, in an orang-outang in Manila. Castellani[6] also found a species of ameba in a liver abscess in a monkey. More recently Musgrave and Clegg[7] reported isolated cases in monkeys which were used for experimental purposes, and Macfie[8] a case in a monkey (Ceropithecus petaurista) which had served as a reservoir for human trypanosomes.

Aside from the amebic dysentery of man the only disease in which an ameba is supposed to be the etiologic factor is the so-called infectious enterohepatitis of turkeys, which, according to Theobald Smith,[9] is caused by Ameba meleagridis.

In the outbreak here described 8 animals out of a total of 15 exposed succumbed, and of 9 showing symptoms only 1 recovered.

THE CHARACTERISTICS OF THE AMEBAS

No special attempt was made to determine the species of the amebas concerned in this outbreak. The ameboid forms were especially numerous in liver abscesses, but could also be readily found in intestinal matter, the encysted forms being more numerous in the latter. In the vegetative stage they showed very active protoplasmic changes when taken fresh from liver-abscess pus, or from fecal material. This movement, which was apparent for a period of from 24 to 36 hours after the death of the animal, even without the aid of a warm stage, consisted of successive rearrangements of protoplasm without noticeable progressive movement across the microscopic field. Pseudopodia of various forms and lengths were extended from the surface and into these the protoplasm flowed, the nucleus changing its position simultaneously. On the completion of a movement the tendency was to return to a circular form about 30 microns in diameter. Absence of locomotion was probably due to the fact that the fluid medium in which the amebas were examined afforded no support for the pseudopodium.

[6] Parasitology, 1908, 1, p. 101.
[7] Rep. Philippine Is., Interior Dept., Bur. Govt. Lab., No. 18, 1904.
[8] Trop. Med. and Parasit., 1915, 9, p. 507.
[9] Bull. U. S. Dept. Agr., Bur. An. Ind., No. 8, 1895.

At times, on one side of the cell body, the protoplasm waved in one direction. The waves were started at one pole; a broad rounded pseudopodium was violently thrown out and whipped to the opposite pole, and the process repeated very rapidly for a period of several seconds or more. In some instances the protoplasm was clear and transparent, showing the circular nucleus plainly, but no granules or

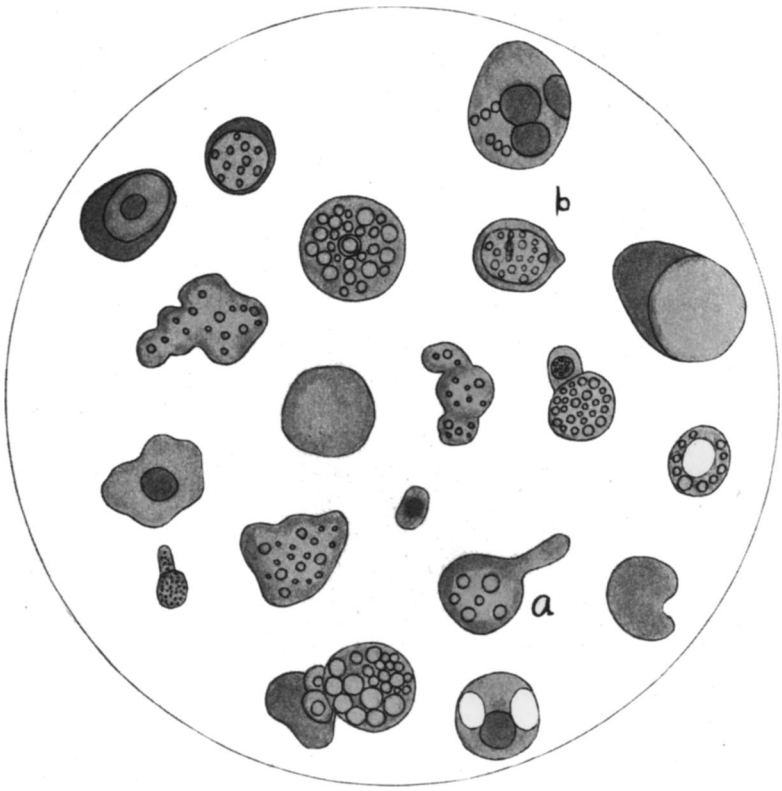

Fig. 1. Amebas in vegetative and encysted forms. (a) Vegetative. (b) Encysted forms. × 250.

foreign bodies. In other cases practically the entire interior was granular and appeared to contain several vacuoles, apparently noncontractile.

During the course of microscopical observations of fresh preparations from the several cases, the amebas gradually lost their motive power, assumed a roughly circular form, became more granular,

decreased in size, and appeared to be encysting. In the encysted stage 2 distinct areas were to be seen; the central portion showed a granular arrangement, was circular in outline, and surrounded by a clear transparent zone, which did not take up ordinary stains readily. In stained tissue sections containing the vegetative forms the nucleus appeared surrounded by a narrow stained band which separated it from the remainder of the cell body.

In feces examined after being voided by sick animals the vegetative forms present were sluggish, and the motility was of short duration, whereas material from liver abscess or intestinal exudate examined from 24 to 36 hours after the death of the animal was found to contain amebas in very active, tho short-lived, motility. This would indicate that the amebas passed with the feces were on the point of encysting in the lumen of the intestine, while those removed from the tissue after death were in an earlier stage of the life cycle, and had maintained themselves for a considerable period in the dead animal matter. The preponderance of encysted forms in the feces supports this view.

HISTORY OF THE OUTBREAK

In July 1915, 3 spider monkeys (Ateles ater) were received at the National zoological park from a dealer who had had them in his possession for some time. During September, 2 more were received, these from Colombia. There were 8 in the shipment, the other 6 being distributed among customers by the dealer. None has been reported sick except those at the National zoological park, and these togethe with the original 3 showed no signs of illness until December. On October 9, 2 spider monkeys were received from Salvador, and on October 26, 3 from Colombia. December 5, 5 gray spider monkeys, also from Colombia, were received. The animals were placed together in a large exhibition cage provided with facilities for exercising. One of the monkeys received October 26 was ill, showing symptoms, according to the keeper, similar to those later manifested by the others. In appears probable that this monkey carried the infection, altho the assumption can not be definitely proved.

The animals ranged in age from 2 to 5 years. In the normal state they were very active and while well filled out and muscular, they showed no tendency to fat-formation. These monkeys do not breed in captivity. Their natural habitat is in Central America, in the region extending from Nicaragua to Colombia, inclusive. They live in high trees and are found at any elevation between sea level and 7,000 feet altitude. In the wild state the food consists of fruits, insects, birds' eggs, young birds, leaves, bark, and seeds. At the National

zoological park the food consists of evaporated milk and raw egg daily, cooked rice, baked beef twice a week, wheat one day and sunflower seed the next, bananas, apples, beets, carrots, and sweet potatoes, both raw and cooked.

Since the date of the introduction of the disease among the monkeys is uncertain, the period of incubation cannot be defined. The first death occurred on November 21, 1 of the Salvador monkeys dying of nephritis, 44 days after arrival. The first death from amebic dysentery was on November 26, the animal that was sick on arrival being the first to succumb. Between November 26 and February 24, 8 animals died of dysentery; 1 had been very ill for a month and had apparently recovered, the only one to recover after showing symptoms of disease. Of the 5 monkeys placed in the cage on December 5 none contracted the disease, altho they were exposed for some time.

The floor and the interior of the cage, which were washed daily, were perfectly sanitary. However, there was ample opportunity for infection to spread from one to another through close association, and through contamination of food and water in the cage by the affected ones. Furthermore, it was observed by the attendants of the monkey house that the spider monkeys in that particular cage developed a depraved appetite for the feces which they evacuated, and as this is somewhat of an unusual occurrence among monkeys, it would readily explain the cause of the rapid spread of the disease.

TRANSMISSION EXPERIMENTS

To determine the relationship of Ameba ateles to the tropical dysentery of man, an attempt was made to transmit the disease to cats, since it is generally understood that these animals are susceptible to Ameba histolytica.

Two grown cats and 2 kittens were fed for 30 days with material from the intestines of monkeys dead of dysentery, and with feces from 2 sick monkeys. The material, which was known to contain the parasite, was fed while comparatively fresh, and also after it had been retained for several days at room temperature to promote encystment. Mixed with the food of the animals it was taken readily. The cats remained healthy, showing no indication of diarrhea, except for an occasional temporary looseness in 2 of them, apparently from other causes. Amebas were not found in the feces and at the expiration of 30 days of feeding, autopsies failed to reveal any lesions in the intestine or in other internal organs.

These negative transmission experiments suggest that the parasite found is of a different species from that in man, and that it is specific for the spider monkey, particularly since the cats cohabitated in the

same cage with sick monkeys, and were afforded ample opportunity for the ingestion of encysted forms of the parasite. The results, however, do not warrant any conclusions as to the pathogenicity of this parasite in cats, since failure of transmission to a limited number of animals is not sufficient evidence, various investigators having found that the transmission of amebic dysentery to cats is not invariably successful. No other species of monkeys kept in other cages of the monkey house became affected, altho they were in close proximity. However, the exposure was likely very slight. There is always a possibility, however, of parasitic organisms' being carried by attendants on cleaning utensils, unless unusual precautions are taken.

SYMPTOMS

The first symptom noticed was a tendency to assume the resting attitude. The affected animal sat upright with the head pressed down between the hind legs and the long tail curled around the body. Marked dejection, stupidity, and lack of interest were evidenced. The temperature remained practically normal and the appetite, while diminished, was fairly good up to the time of death. The animals decreased in weight during the period of illness, but were not markedly emaciated.

The principal symptom was a severe diarrhea. The feces were usually of a fluid consistency, yellowish-gray in color, and fetid, containing at times considerable mucus with yellowish flakes. In the more acute cases the excrement was sometimes blood-stained. At first the diarrhea was intermittent, fairly well-formed stools being passed during intervals, but later the diarrhea persisted till the end. In several cases symptoms were apparent for only 2 or 3 days before death, while in other cases the animals showed signs of illness over a period of from 2 to 4 weeks, at times appearing to have recovered somewhat, only to relapse and die suddenly.

GROSS LESIONS

In the 8 cases in which autopsies were made the cecum and colon were invariably the seat of pathologic changes, the rectum being involved to a greater or less degree. No lesion could be detected macroscopically in other portions of the gastro-intestinal tract. Even in the most gross cecal infection the amebic invasion did not pass beyond the ileocecal opening. Liver abscess was present in 2 cases. The mesenteric lymph glands in the region of the colon were in some instances found to be enlarged and edematous.

In the large intestine, which was approximately 18 inches long in the monkeys under discussion, the lesions were pronounced and distinctive. The appearance of the grossly affected portions of the mucosa was that of a dense corrugated mass of grayish-white mealy-looking necrotic matter. In the advanced cases where the disease had evidently been of long duration, the process had spread to practically the entire membrane. In less affected intestines numerous small ulcers, varying in size from 1 mm. to 1 cm. in diameter, were found well separated from each other. These presented raised irregular borders on which a line of congestion could readily be traced. The depressed centers were occupied by a fairly closely adhering mass of necrotic exudate, which also

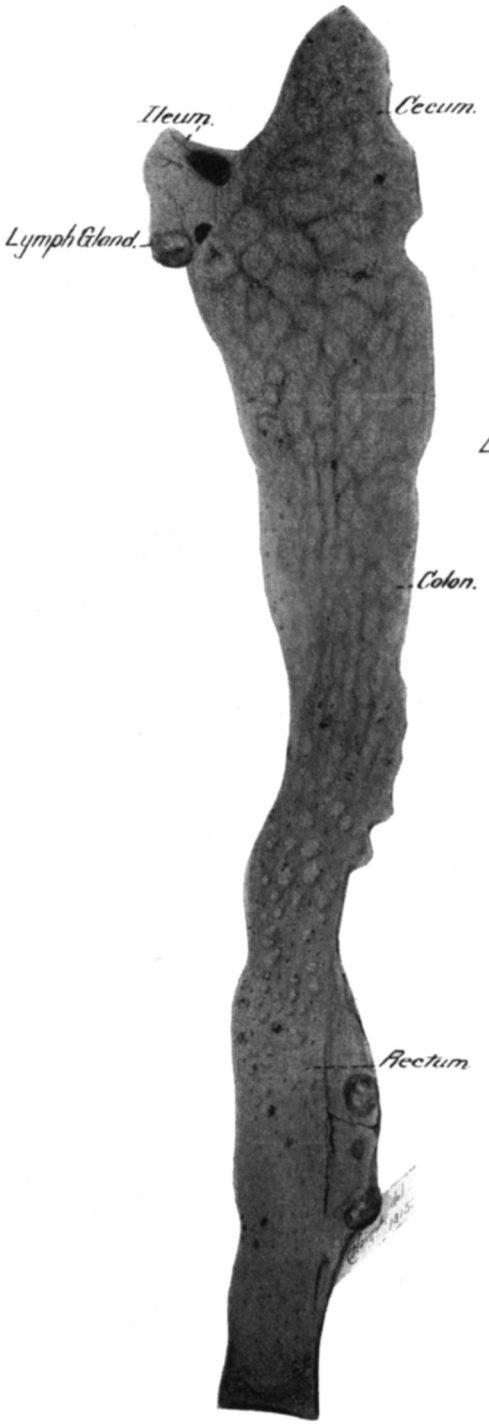

Fig. 2

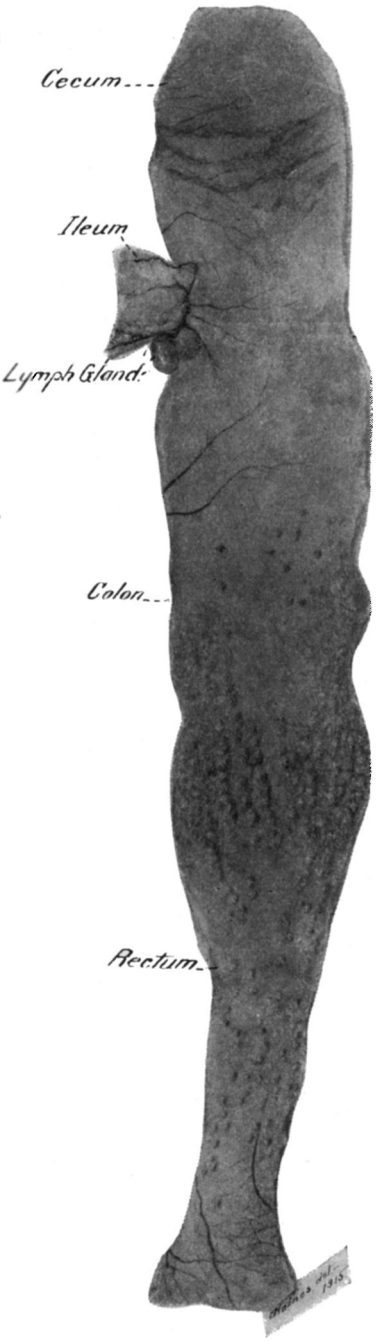

Fig. 3

Fig. 2. Large intestine of a monkey illustrating extensive exudate on the surface of the mucosa of cecum and colon, and ulceration of the rectum. Extensive involvement with diversified and confluent lesions. About one-half natural size.

Fig. 3. Large intestine of a monkey illustrating ulceration of colon and rectum. Cecum covered by a catarrhal exudate. Lesser involvement with ulceration predominating. About one-half natural size.

covered the surface of the ulcer to a height of from 2 to 3 mm. Affected portions of the intestine not marked by ulceration showed a thick covering of an amorphous detritus on the surface of the mucosa. The thickness of the exudate in severe cases gave the cecum and colon a dilated appearance and a semirigidity. The material composing the exudate could readily be scraped off the surface, appearing as a flaky mass easily broken up into fine particles; however, on its deep border it had more of a diphtheritic consistency and had a tendency to remain adherent to the neighboring tissue. Lines or points of deep congestion, or hemorrhage, were of frequent occurrence in the affected parts.

The deep ulcers penetrated beyond the submucosa, but no indication of the condition in the lumen of the intestine was apparent from the peritoneal surface, save where dilation or rigidity of the wall was present.

Abscess of the liver was associated with the 2 cases showing the most extensive intestinal lesions. In one case only 1 abscess was present, while in the other 5 fairly large abscesses and 2 smaller ones were observed. They were characterized by the absence of a well-defined capsule except at the point where the capsule of the liver was in apposition, at which a marked thickening of the latter had occurred. The borders were surrounded by a zone of small necrotic areas of irregular outline. The abscesses were deeply extended and spread out within the interior of the lobes. Their interiors held the remains of liver structure which had not yet undergone complete disintegration, in a network in which pockets and channels were conspicuous. These openings were filled with a grayish-white fluid pus which exerted an appreciable pressure on the abscess border. In fresh preparation of the pus the microscope disclosed numerous amebas showing protoplasmic movement.

HISTOLOGY

Intestine.—The intestinal wall showed variable changes according to the degree of involvement. Ulceration of the mucosa was prominent in places, the position of the glands of Lieberkuhn being occupied by detritus, in which were observed disintegrating mucous cells, lymphoid cells, and amebas. The thickness of the necrotic exudate covering the affected areas was about twice the thickness of the normal mucosa. The necrotic foci were at times partially encapsulated by fibrous tissue and lymphoid cells, especially when the submucosa was involved. The tissue surrounding the foci did not appear to be greatly damaged, altho the immediately adjacent cells stained more diffusely and were more or less separated, indicating an edematous condition of the part. A striking feature of some pieces of cecal tissue was the fact that altho there was a dense exudate covering the entire mucous membrane, the latter revealed marked changes only in its surface cells. These were disintegrated and separated from the basement membrane here and there, while at other points damage was evidenced only by the difference in staining properties. The submucous, muscular, and serous layers of the intestine were not particularly affected except in areas of deep ulceration.

Superficial ulcers manifested a tendency gradually to destroy the adjacent mucosa without causing any accumulation of inflammatory cells, and without a trace of the formation of fibrous tissue. This probably indicated rapid advance without a marked stimulative effect on the protective forces of the system.

In the case of deep ulcerations the necrotic process had involved not only the mucosa, but had penetrated also through the submucosa, muscularis mucosa, and circular muscular layer, and produced distinct degenerative changes in the longitudinal muscular layer. Around the base of the deep ulcers small mono-

nuclear leukocytes were massed in large numbers. A distinct characteristic of deep ulcers was the formation of a dense fibrous wall undermining the mucosa to a considerable distance, and causing a thickened or raised appearance in the ulcered area. The mucosa in the undermined region had in areas almost lost its identity, and tho in adjoining areas the structure was preserved, disintegration changes were quite apparent. The center of the ulcers consisted of an amorphous mass, breaking through the surrounding fibrous wall and resting in the circular muscularis, the surrounding fibers of the latter showing marked degenerative changes to a considerable depth.

In some instances the mucous membrane surrounding the mouth of the ulcer presented a punched-out appearance; in others the membrane was shrunken or erased on one side, while seeming fairly normal on the opposite side in the histologic section.

The histologic picture was characterized by the absence in large part of micro-organisms other than amebas; the absence of the types of leukocytes usually found present in microbic infections; the failure of fibrous capsule to form, except in the submucous layer, where the thickening was marked when ulcers extended into this coat; and by the dense covering of detritus at points where little harm had been done to the underlying mucosa. The absence of pronounced congestion except in superficial areas where microbic activity was more or less evident, was also characteristic, and suggested the specific nature of the amebic lesions.

Amebas were disposed through the proliferated material and were to be found in the disintegrating tissue surrounding the ulcers and in the glands of Lieberkuhn in affected areas.

Liver.—The tissue bordering a hepatic abscess presented numerous necrotic foci varying in size from a 4- or 5-cell area to a macroscopic abscess. The tendency in the larger foci was toward encapsulation. A considerable number of lymphoid cells were observed in the fibrous structure of the wall. The encapsulated mass represented a homogeneous material in which a few lymphoid cells and also amebic forms might be found.

The picture was characterized by the varying degree of the degenerative processes in the infected areas. At points where several amebas were lodged no changes were observed in the staining properties of the surrounding liver cells, altho a distinct separation of the cell columns was evidenced. At points where degenerative changes had progressed to a detritus-formation the amebas were arranged around the periphery of the foci and extended in places beyond the zone into the normal structure. In the case of the more advanced foci a well-defined wall of fibrous tissue had formed, lymphoid cells being present to some extent in the outer border, and at times in the central mass. In some instances the encapsulation was marked and apparently had arrested the amebic progress, as no amebas were visible in the foci.

Between the foci of degeneration the liver cells did not appear to have become at all involved except in the case of those forming the surrounding layer, and even here there was no marked change unless the area was partly inclosed by 2 or more foci.

Isolated amebas were found in the midst of normal hepatic cells at a distance from visible lesions, but it is probable that these had migrated from closer lesions not revealed in the particular tissue section.

Congestion was in evidence, the small vessels in the invaded areas showing a noticeable fullness. However, no hemorrhages were observed in the tissue examined.

CASE 1

Carcass somewhat emaciated. Left lung congested in anterior lobe. Right lung slightly congested. Considerable clotted serous exudate in pleural cavity. Spleen enlarged to twice its normal size.

The anterior surface of the liver contained an abscess covering about one-third the area of the principal lobe. The apex of the abscess pointed to the diaphragm, to the fibrous part of which it was firmly attached over an area three-quarters of an inch in diameter. Around this adhesion the abscess-formation was well marked beneath the capsule, which had a thickened appear-

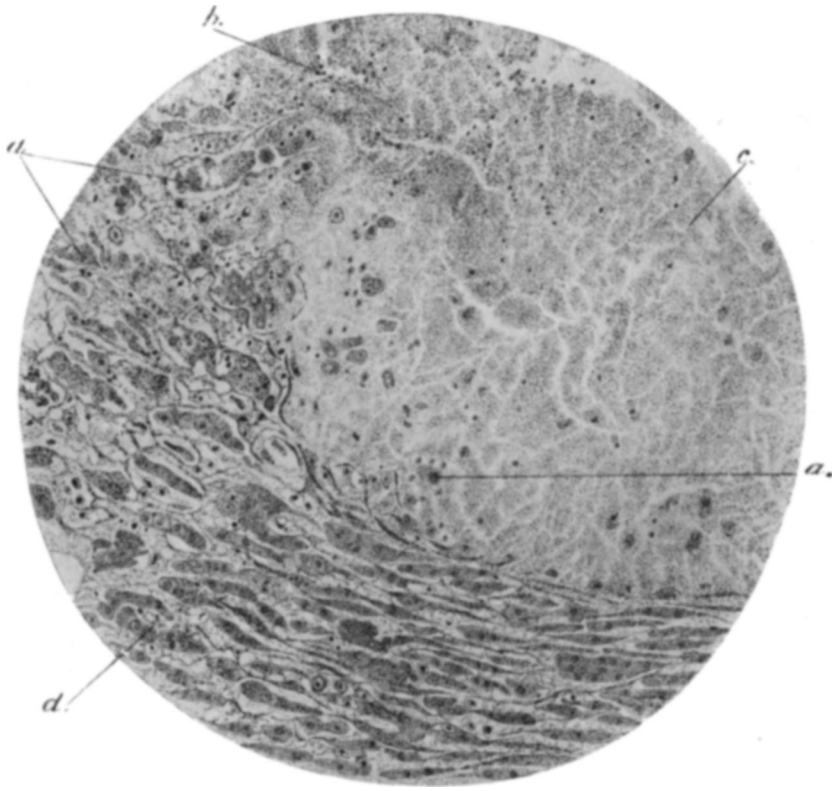

Fig. 4. Section of the liver of a monkey illustrating the edge of small necrotic foci. (a) Amebas. (b) Lymphoid cells. (c) Necrotic detritus. (d) Liver cells. × 125.

ance. Surrounding the border of the large abscess were numerous small necrotic areas varying in size from one to several millimeters in diameter. When the adherent diaphragm was cut away, a grayish-white pus was released, proceeding from several well-defined channels within the degenerated liver structure. Microscopical examination of this pus in the fresh state revealed numerous amebas undergoing active changes in the form of their protoplasm. On cut section the abscess proved to be deeply extended in the body of the lobe, undermining the superficial structure.

Entire cecum and nearly the entire length of the colon covered on the surface of the mucosa with necrotic material of a grayish-white color. Exudate from 2 to 3 mm. in thickness, of mealy appearance, and thrown into longitudinal and horizontal folds; of a granular consistency in its deepest layer, approaching the diphtheritic type of exudate. The extensive necrotic formation gave the intact cecum and colon a distended appearance and semirigid position, which directed attention to their abnormal condition before an incision through their walls was made. The process did not extend to the ileum, altho the ileocecal valve was involved. In the posterior portion of the colon and the anterior part of the rectum ulcers from one to several millimeters in diameter were found, isolated and numerous. These ulcers had a raised hemorrhagic border, were irregular in shape and covered by the same type of exudate as that previously described. Hemorrhagic points observed here and there throughout the affected area.

Microscopically the fresh cecal material showed the presence of motile and encysted amebas.

CASE 2

Carcass in fair condition. No lesions apparent in internal organs other than cecum, colon, and rectum.

Cecum and colon dilated and fairly rigid. On being incised the mucous membrane found in a condition similar to that of Case 1, the thickened mass of exudate being strikingly prominent in spots, but not as widely distributed over the surface. Congestion relatively marked over the entire area of the mucous membrane of the colon and rectum. Mesenteric glands in the affected region edematous.

Amebas present in the cecal material.

CASE 3

Carcass emaciated. Internal organs other than large intestine and liver normal.

Distributed through the liver were 5 abscesses varying in size from one-half to three-quarters inch in diameter, and 2 smaller abscesses. These which did not appear to be well encapsulated, were surrounded by zones of minute areas of degeneration. Capsule of the liver noticeably thickened over the abscesses, preventing rupture into the abdominal cavity.

Mucous membrane of the cecum and colon ulcerated and covered in large areas by the heavy uneven-surfaced necrotic material. Numerous small ulcers with raised congested edges scattered in the less affected regions. Mesenteric lymph glands enlarged and congested.

CASE 4

Carcass somewhat emaciated. Internal organs other than large intestine apparently normal.

Mucous membrane near the blind end of the cecum thickened, giving indication of early ulcer-formation. Surface covered by a thin deposit of necrotic matter. Mucosa of the posterior portion of the colon covered with small ulcers 3 mm. in diameter, isolated from each other and showing prominent raised hemorrhagic borders. A dense grayish-white exudate capped each ulcer. Between the ulcers mucosa normal. Anterior extremity of the rectum also ulcerated, but ulcers less numerous and more widely separated. Several healing ulcers obscured, raised above the surrounding membrane and containing a central

depression. Some exudate material still clung to the surface. Mesenteric glands along the affected area edematous.

Amebas present in smears of cecal material.

CASE 5

Animal received from the zoological park in a sick condition; droopy; sat huddled up and inattentive. Diarrhea a prominent symptom, the feces liquid with small amount of solid material, and yellowish-gray in color; amebas present. Appetite good until the day before death. Depression became more pronounced, death following a period of 12 days' noticeable illness.

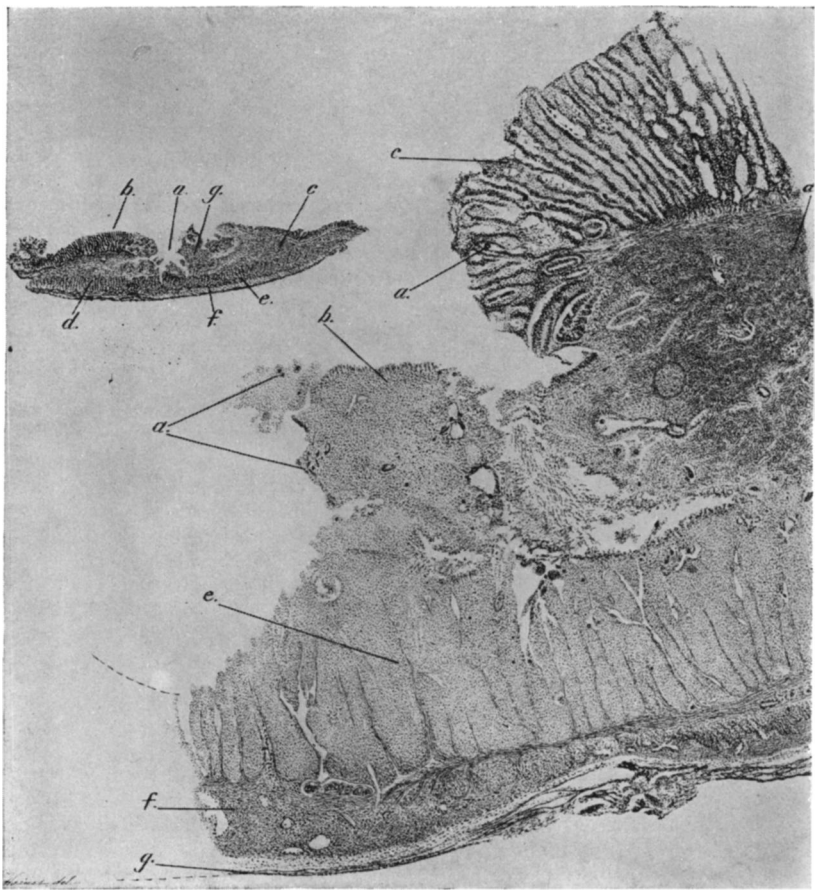

Fig. 5. Vertical longitudinal section of colon illustrating deep ulcer. (a) Ulcer. (b) Mucosa. (c) Dense fibrous tissue replacing submucosa. (d) Circular muscular layer. (e) Longitudinal muscular layer. (f) Serous layer. (g) Detritus. × 7.

Fig. 6. Vertical longitudinal section of colon illustrating edge of deep ulcer. (a) Amebas. (b) Necrotic detritus. (c) Mucosa. (d) Fibrous wall. (e) Circular muscular layer. (f) Longitudinal muscular layer. (g) Serous layer. × 30.

Carcass in fair condition. The cecum and colon showed corrugation of the mucous membrane in patches associated with inflammatory areas. Ulceration and exudate-formation in places, but not as extensive as in the previous cases. Exudate material revealed amebic forms.

CASE 6

Carcass emaciated to some extent. Cecum, colon, and anterior rectum presented the characteristic ulceration of the mucosa observed in previous cases. The lesions were developed in patches and in areas of diffuse single ulcers. Amebas in the cecal exudate.

CASE 7

Carcass showed no fat. All organs except large intestine apparently normal. Cecum, colon, and first portion of rectum showed diffuse areas of congestion associated with small patches of yellowish exudate, giving the appearance of dull sulfur-like deposits scattered over the surface of the mucosa. The intestine was practically free from feces, but a small amount of viscid material coated the cecum, the wall of which appeared thickened, tho not thrown into ridges. Membrane of the colon and rectum smooth with no marked ulceration, but with numerous congested areas and necrotic patches.

The contents of the intestine, especially the sulfur-like grains of exudate, showed numerous amebas in active protoplasmic movement, also encysted forms. No progressive movement across the field of the microscope, but pseudopodia of different forms thrown out, the nucleus changing its position rapidly as the protoplasm flowed into the protruded capsule.

CASE 8

Animal had been sick for 2 weeks before being brought to the laboratory. Condition grew worse daily; the feces were liquid and became blood-stained at times several days before death, which occurred in 12 days. At autopsy considerable emaciation evidenced.

All internal organs apparently normal except the large intestine; diffuse ulceration throughout its length. Colon conspicuous by its hemorrhagic appearance, almost the entire surface of the mucosa being deeply reddened. This inflammatory condition entirely superficial, involving the mucous membrane alone. The picture represented extreme congestion, the earlier cases being of the exudative type and showing congestion only in small lines around ulcers, or in small areas. In this case the ulceration was not marked and little exudate was apparent.

Altho motile forms of the ameba were numerous in fecal material for several days before death, only encysted forms were observed immediately after death.